REMARKS

Claims 1-21 are pending in the application. Claims 17-21 have been withdrawn as directed to non-elected subject matter.

Claim 14 was objected to as depending on a rejected base claim. Claim 14 has been rewritten in an independent form. No new matter is added by virtue of this amended and its entry is respectfully requested.

Claim Rejections Under 35 U.S.C. § 102

Claims 1-12, 15 have been rejected under 35 U.S.C. § 102 (e) as being anticipated by Wang *et al* (US 6,063,259 A). Applicants respectfully traverse.

Applicants' invention is directed in part to a circuit board biosensor apparatus wherein the apparatus comprises reference electrodes (see, for example, page 6, lines 25-30; page 7, lines 22-34; page 20, lines 22-33 through to page 21, lines 1-2; page 22, lines 21-34 through to page 24 lines 1-21; page 28, lines 7-33 through to page 29, lines 1-7; Examples 4.1 - 5.13); a plurality of nucleic acids attached thereto (see, for example, page 8, lines 13-29; page 10, lines 8-33; page 18, lines 19-33 through to page 20, lines 1- 15; page 21, lines 19-33 through to page 24, lines 1-26; page 26, lines 11- 32 through to page 27, lines 1-19; Examples 4.1-5.13); a means for measuring current (see, for example, page 7, lines 12-21; page 11, lines 1-15; page 18, lines 7-18; page 19, lines 29-33 through to page 20, lines 1-2; page 22, lines 9-20; page 27, lines 20-33 through to page 29, lines 1-7). The current is produced by the hybridized electrode bound nucleic acid segments and nucleic acid target sequences when an electric potential is applied.

Applicants also teach a pulse amperometric monitor for the electrochemical detection of nucleic acid sequences (Claims 4, 6 and dependent claims therefrom). See, for example, Figures 5, 6, 7-10, 12, and 15-18 showing data obtained with a pulse amperometric monitor and the text of the instant application on page 21, lines 2-33 through to page 22, lines 1-34. Also described is an amperometric monitor for the electrochemical detection of nucleic acid sequences that

comprise pulse and intermittent pulse modes of operation. See for example, page 51, lines 1-33 through to page 56, lines 1-15; Figure 7 shows the nature of the varied modes of applying potential to the sensors.

In contrast to applicants' invention, Wang *et al.*, does not teach or disclose the use of an electrochemical apparatus for establishing an appropriate potential on an electrode surface so that an electrical current can be generated and used for nucleic acid and/or protein, polypeptide, peptides or protein nucleic acid detection. (See for example, page 7, lines 6-11 of the instant application. In contrast, in Wang *et al.*, measurement of changes in the electrochemical potential of a sensor can be used to detect nucleic acids, and not, as in the instant invention, whereby the current generated at a sensor is used to detect nucleic acids. In other words, Wang *et al.*, is directed to potentiometric methods, procedures, and end results which are fundamentally different. Potentiometric detection of nucleic acids does anticipate in any way the possibility of amperometric detection of nucleic acids. Wang *et al.*, teaches that the potential at the sensor surface changes when materials (specifically guanine residues of nucleic acids) that are absorbed or immobilized on the sensor are chemically oxidized. See, for example, Col. 6, lines 3-31:

> In both methods of the invention, potentiometric stripping analysis (PSA) is employed to follow the oxidation of either the adsorbed nucleic acid species (Method 1) or the hybridization events (Method 2). In particular, constant current potentiometric stripping analysis (ccPSA) is used for measuring remarkably low levels (picogram (pg)) levels of nucleic acids at the carbon-based electrodes. Selected parameters of the fabrication process and the PSA operation allow convenient monitoring of sub-microgram per liter nucleic acid concentrations. Detection limits are 3 mg/L for tRNA, 25 mg/L for ssDNA, and 30 mg/L for dsDNA. Unlike analogous voltammetric monitoring of the adsorbed nucleic acid, which suffers from a large background current at the high potential associated with the oxidation of the guanine moiety, the computerized PSA operation offers a nearly flat baseline, thus enabling substantially lower detection limits. As desired for single-use applications, such operation eliminates the need for the previously-used (toxic) mercury-drop electrodes and related time-consuming deaeration steps. (Emphasis added).

Wang *et al.*, do not measure current generated at the sensor, as taught by Applicants. The current in the instant application is produced by the hybridized electrode bound nucleic acid

segments and nucleic acid target sequences when an electric potential is applied. Wang *et al.*, do not teach or disclose the instant invention of a means whereby a current can be generated and measured that is proportional to the amount of a specific target nucleic acid that is absorbed or immobilized on an electrode surface.

The Examiner's allegations that Wang *et al.*, is inclusive of claims 4-6 is therefore, incorrect. Furthermore, it is well known to any one of ordinary skill in the art that there are fundamental differences between potentiometric assays and amperometric assays. Potentiometric stripping analysis (PSA) does not measure current. It involves a preconcentration step and a subsequent striping step. Preconcentration can involve adsorption or electrodeposition, or other means of capturing the analyte/target on the electrode surface. Potentiometric stripping involves monitoring changes of the potential of the electrode as the captured analyte/target is allowed to undergo oxidation or reduction. The oxidation or reduction can be accomplished by chemical means, i.e. using a stripping reagent, or by electrochemical means. The potential of the electrode remains relatively constant as long as the analyte/target species are still present on the electrode surface. When the analyte/target is completely oxidized or reduced the potential of the electrode changes abruptly. The amount of time or charge needed to reach this point is proportional to the amount of the analyte/target preconcentrated on the electrode and consequently to the amount or concentration of the analyte/target in the sample. Wang *et al.*, state in col. 10, lines 9-19:

> In testing, it was also found that the sensor response toward nucleic acids is strongly dependent upon the pre-concentration period. FIG. 4 displays stripping potentiograms for 5 mg/L dsDNA after different pre-concentration periods ranging from 1 second (a), 60 seconds (b), 90 seconds (c), 120 seconds (d), 150 seconds (e) and 180 seconds (f). Other than pre-concentration periods, other conditions used were those detailed for obtaining the plots of FIG. 2. Also shown as insets are plots of the stripping time versus the accumulation period for 5 mg/L dsDNA (a), ssDNA (b) and tRNA (c). (Emphasis added).

Potentiometric stripping that involves a constant current is called the constant current Potentiometric Stripping Analysis (ccPSA). A variable potential is applied to the electrode to force a constant current. This can be done in a form of current pulses and the potential (E, signal) is measured between the pulses. The measured potential (E) is plotted as a function of

time (t) and the data is graphed as E-t curves. The quantization is usually based on the amount of time required for the potential to change.

The principles of amperometric measurement, and in particular the intermittent pulse amperometry (IPA) of the instant invention claims, are completely different. In amperometric measurements it is the current generated at the electrode that is measured. In this measurement a potential is applied on the electrode and the resulting current is measured. The measured current is proportional to the concentration of species that can be oxidized or reduced by the applied potential. In contrast to the ccPSA described above, there is no measurement made when the potential is not applied to the electrode. In the instant invention, amperometric determinations of nucleic acids, the potential is controlled and the current measured (See, for example, Claim 1 of the instant application).

Yet another important distinction between ccPSA (Wang *et al*) and the instant invention lies in the sensitivity of detection achievable. By monitoring only the potential at the electrode surface, which is determined in Wang *et al.*, by the environment and abundance of guanine residues, Wang *et al.*, claims detection of one nanomole of a target nucleic acid (i.e. 600,000,000,000,000 molecules). See, Wang *et al.*, column 6, lines 12-13. In contrast, using the disclosed amperometric measurements, Applicants have selectively detected nucleic acid targets down to 30,000 molecules. See, for example, page 22, lines 17-20 of the instant application. This extreme difference in sensitivity makes it possible for the instant amperometric approach to be used in many human and environmental health applications where potentiometric assays have no possibility of being used. In summary, the instant invention is novel over Wang *et al.*

The Examiner also alleges that: Wang *et al.*, discloses DNA and RNA spontaneously immobilizing on the electrode (Col. 5, Wang *et al.*); that Method 2 by Wang *et al.*, (col. 6, lines 45-55) describes an electric potential applied to the working electrode when the attached nucleic acid segments hybridize to nucleic acid targets; operation of microfabricated DNA strips combined with a hand-held, battery operated potentiometric stripping analyzer is inclusive of claim 7; built-in software controls the entire sequence of events (Wang et al., col. 7, lines 58-67)

is viewed as being inclusive of the single key start operation of claim 11 and the microprocessor of claim 10.

Applicants respectfully traverse. In view of the afore going reasons, Applicants submit that Wang *et al*. do not teach or disclose the instant invention.

Applicants teach (Claim 5) a pulse amperometric monitor that simultaneously detects and quantifies levels of a plurality of targets on electrode arrays where dual sensors can be used as elements of the array. The basic Electrode Design is described in section 3.2.1 of the application, with single and dual sensors shown in Figures 2A and 2B, respectively. The use of pulse amperometric monitors with dual sensors for detection of nucleic acid sequences is described on, for example, page 63, lines 7-33 through to page 65, lines 1-29.

As discussed above, applicants measure the current produced by the capture of a target nucleic acid. That is, the current is target induced and not applied by the apparatus. Moreover, the small, portable instrument that applies potential to a working electrode is the same instrument that transfers, records, analyzes and/or displays the current generated by the electrochemical assays as taught by applicants. (See for example, page 18, lines 7-18; page 19, lines 1-8; page 28, lines 7-30). Applicants apparatus is also tailored as a small, portable instrument that applies potential to a working electrode is the same instrument that transfers, records, analyzes and/or displays the current generated by the electrochemical assays. (See for example, page 19, lines 1-8).

Furthermore, specific differences between Wang *et al*., and the instant invention are as follows: (1). Potentiometric stripping and amperometric measurements are two fundamentally different electrochemical techniques. (2). Wang *et al*., does not teach or disclose a sensor and methods of detecting current produced by the hybridized electrode bound nucleic acid segments and nucleic acid target sequences when an electric potential is applied. In contrast, Wang *et al*., refers to a method based on a direct detection of nucleic acid targets which is *via* oxidation of one of the four nucleic acids- guanine, while, in contrast, the instant invention detects current generated from captured targets. (3). The instant invention is non destructive with respect to the

target because it involves an electrochemical reduction of a reagent and not the target nucleic acid, whereas, Wang *et al.*, is based on an electrochemical oxidation of the target nucleic acid, which alters the chemical nature of the target. (4). In its highest sensitivity embodiment, the instant application discloses signal amplification via the use of enzyme labels on captured targets. No such teachings or disclosure is found in Wang *et al.* (5) Establishing a system of one or a plurality of electrodes where changes in potential are measured does not in anyway suggest or imply to one skilled in the art that a system can be generated in which the current generated on one or a plurality of electrodes is measured and used to monitor the level of a nucleic acid target sequence. (6). The detection sensitivity achievable with the instant invention is at least 7 orders of magnitude greater than that achievable with that the potentiometric approach described by Wang *et al.* (7). Wang *et al*, do not teach or disclose a single key start operation. Consequently, supports, labels, and methods of applying a voltage waveform effective to trigger light emission (for an optical detection system) differ greatly from the application of specific electrical potentials effective to result in current generation when a target nucleic acid is captured at a surface, as in the instant invention. Applicants submit that Wang *et al.*, does not teach each and every limitation of applicants invention.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. § 102(e) rejection as it applies to claims 1-12 and 15.

Claim Rejections Under 35 U.S.C. § 103

Claims 1-13, 15, 16 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Wang *et al.*, (U.S. 6,063,259 A) in view of Murtagh (5,518,901).

Applicants respectfully traverse.

Wang *et al.*, has been discussed in detail above and for the sake of brevity will not be repeated here. As discussed above, applicants measure the current produced by the capture of a target nucleic acid. That is, the current is target induced and not applied by the apparatus.

Moreover, the small, portable instrument that applies potential to a working electrode is the same instrument that transfers, records, analyzes and/or displays the current generated by the electrochemical assays as taught by applicants. (See for example, page 18, lines 7-18; page 19, lines 1-8; page 28, lines 7-30). Applicants apparatus is also tailored as a small, portable instrument that applies potential to a working electrode is the same instrument that transfers, records, analyzes and/or displays the current generated by the electrochemical assays. (See for example, page 19, lines 1-8). Applicants further teach, the material needed to for the electrodes, the nucleic acid sequences, the attachment of the nucleic acid probes to the electrodes, the quantity of capture nucleic acids e.g. number per area (surface of electrode can be as small as 0.001 mm^2 to about 100 mm^2; page 8, lines 4-5), the pattern of nucleic acids on the electrodes, the length of nucleic acid capture sequences, the solutions needed to measure the electric current, especially in view of detection of one nucleic acid sequence difference (see, for example, detection of single nucleotide polymorphisms on page 31, lines 8- 32 through to page 33, lines 1-28) and the like.

On page 7 of the Office Action, the Examiner asserts that:

> Murtagh discloses method for detecting the presence of nucleotide sequence within a double stranded DNA in a sample comprising the step of digesting the double stranded DNA with exonuclease which converts at least a portion of the double-stranded DNA to single-stranded DNA.

Applicants respectfully traverse. Applicants teach use of a 5'-phosphate modified reverse primer during a PCR step to generate double stranded PCR products that can subsequently be made into single stranded DNA by digestion with an exonuclease that acts on the 5'-phosphate modified strand. The forward primer used in the PCR may be optionally modified as well, so that the single-stranded material remaining after exonuclease digestion is prepared for a) binding to the sensor surface and b) hybridization with a detector probe that has high selectivity for the nucleic acid target sequence of interest. (see, for example, page 14, lines 7-24; page 30, lines 3-33 through to page 31, lines 1-7).

Murtagh presents data on use of exonuclease as an enzyme reagent only with regard to improved use of the polymerase chain reaction (PCR) technique whereby many copies of a segment of a target nucleic acid can be obtained. In the instant invention, Applicants teach the use of the exonuclease reaction only in specific applications of amperometric detection methodology. In these applications the double-stranded product of a PCR reaction is treated with the exonuclease enzyme with the specific aim of making it single stranded. Once it is single-stranded, it can be hybridized with a detector probe and then electrochemically detected. In contrast to the instant invention, Murtagh uses exonuclease in generating an improved PCR reaction, while Applicants teach the means whereby a PCR reaction product can be selectively detected via amperometric electrochemical detection.

In summary, Applicants teach measuring the current produced by the capture of a target nucleic acid. That is, the current is target induced and not applied by the apparatus. Neither Wang *et al.*, alone or in combination teach Applicants invention.

Applying Wang *et al.*, which does not teach measuring the current produced by the capture of a target nucleic acid to Murtagh which is directed to identifying DNA in a sample comprising DNA which is the product of a DNA amplification technique and which does not teach or disclose the sensor and methods of Applicants invention does not make the instant application obvious. Mutagh state in col 4, lines 24-39:

> The present invention provides a method of detecting the presence of a nucleotide sequence within a double-stranded DNA in a sample comprising: a. digesting the double-stranded DNA with an exonuclease which converts at least a portion of the double-stranded DNA to single-stranded DNA, b. binding the single-stranded DNA with a nucleic acid probe which selectively hybridizes with the single-stranded DNA, and c. detecting hybridization between the single-stranded DNA and the nucleic acid probe, the existence of hybridization indicating the presence of the nucleotide sequence within the double-stranded DNA in the sample. The present invention further provides a method of detecting the presence of a nucleotide sequence in a sample comprising DNA which is the product of a DNA amplification technique. (Emphasis added).

Furthermore, Murtagh uses a colorimetric assay which is routine to one of ordinary skill in the art. Detection of color changes as opposed to measuring an electric current produced by hybridizing molecules, would inherently require significantly different supports, labels and methods. There is no motivation provided in either references, nor a reasonable expectation of success to one of ordinary skill in the art to combine the teachings and arrive at the instant invention. Neither Wang *et al.*, alone or in combination with Murtagh teach nor disclose that a sensor and means thereof for the detection of target-dependent electrical currents (not color) generated by targets selectively captured on electrodes. Both cited articles fail to disclose means to generate and detect currents associated with captured nucleic acid targets.

For at least the reasons given above, Applicants respectfully submit that Claims 1-13, 15, and 16 and dependent claims therefrom are allowable over the cited references of record. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

Applicants note the Examiner's objection to claim 14, which the Examiner states

CONCLUSION

Applicants respectfully request entry of the foregoing remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-16 define patentable subject matter and is in condition for allowance. Accordingly, Applicant respectfully requests allowance of these claims.

This response is being filed within the shortened statutory period and thus believe that no fees are due. Although, Applicants believe that no extensions of time are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Respectfully submitted,

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